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## The prime-boost strategy: exciting prospects for improved vaccination

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**E**ffective vaccination strategies are urgently required for several viruses, including human immunodeficiency virus (HIV), for which conventional and molecular approaches have not been highly successful. Recent advances in our understanding of the roles played by different T-cell populations has allowed a more rational approach to this problem. It now appears that protection against infection by many agents, particularly viruses, requires the generation of potent cell-mediated immunity (CMI). CMI responses are typically mediated by CD8-bearing cytotoxic T lymphocytes (CTLs) and CD4-bearing T helper (Th) cells of the Th1 phenotype, both of which express a variety of effector functions<sup>1,2</sup>. Vaccines capable of eliciting strong CMI are now thought to hold the most promise for control of infections by many intracellular agents, including HIV (Refs 3, 4).

Vaccines that mimic the antigenicity of infectious organisms may ultimately prove to be the most effective strategy for achieving such protective immune responses. In this respect, DNA vaccines<sup>5</sup> and attenuated recombinant viral vectors, particularly those that are, in some way, unable to replicate in mammalian hosts [e.g. fowlpoxvirus (FPV)<sup>6</sup> and modified vaccinia virus Ankara strain (MVA)<sup>7</sup>] have significant advantages over alternative immunization strategies. These constructs are replication deficient, non-integrating, stable and are relatively easy

*Unprecedented levels of cell-mediated immunity can be stimulated by the consecutive use of DNA vaccines and attenuated poxvirus vectors encoding similar heterologous antigens. This may offer a means of preventive vaccination against diseases for which no effective treatment is available.*

to prepare. They are also capable of inducing excellent CMI and humoral immune responses, largely due to their effective delivery of heterologous proteins into the antigen-processing pathways of transfected or infected cells. There are now numerous animal studies demonstrating the immunogenicity of DNA vaccines and attenuated viral vectors and, in some cases, work has progressed to Phase I studies in humans. Yet, despite encouraging early results, the levels of specific immunity induced by these vectors has generally been insufficient to afford protection against challenge with highly pathogenic organisms.



### Prime-boost vaccination

Recently, a novel strategy involving priming with DNA vaccines and boosting with FPV or MVA, each expressing similar antigens ('prime-boost'), has resulted in the generation

of unparalleled levels of specific immunity and, in some cases, afforded protection against infectious agents that currently pose great problems for vaccine development.

The first demonstration of greatly heightened immunogenicity following the consecutive use of DNA and an attenuated virus was reported using vectors encoding the hemagglutinin (HA) gene of influenza virus. Mice primed with DNA and boosted two weeks later with a recombinant FPV produced extremely high levels (over 1 mg ml<sup>-1</sup>) of anti-HA serum antibodies, predominantly of the IgG2a isotype, unlike animals given either of the vectors alone<sup>8</sup>. Production of the IgG2a subclass is normally associated with interferon  $\gamma$  (IFN- $\gamma$ ) secretion and the development of Th1-type immune responses. The DNA/FPV-vaccinated mice were protected against challenge with homologous influenza virus and, crucially, were also resistant to challenge with a recombinant vaccinia virus (rVV) encoding the same HA antigen<sup>9</sup>. In our model, T cells and their secreted antiviral factors are known to be essential for clearance of VV. Tetramer binding studies showed that up to 30% of circulating CD8<sup>+</sup> T cells were specific for the immunizing epitope (M. Estcourt et al., unpublished). Preliminary studies in nonhuman primates using DNA/FPV prime-boost vaccination generated responses that were fully protective against challenge with HIV-1 (Ref. 10). Protection was achieved using DNA vaccines and FPV

vectors, which both encode env and gag proteins of HIV-1, and was associated with potent, specific CTL and Th1-cell responses. Interestingly, only low levels of specific antibodies were raised following DNA vaccination, and these declined markedly following FPV boosting, despite a marked Th-cell proliferative response against both encoded HIV proteins, indicating a strongly biased Th1-type immune response.

In a subsequent study, macaque monkeys were primed with DNA and boosted with recombinant FPV, each encoding similar HIV and simian immunodeficiency virus (SIV) antigens<sup>11</sup>. Using this strategy, the monkeys were protected against challenge with a strain of simian-human immunodeficiency virus (SHIV HXBC2) in the absence of specific neutralizing antibodies. Four out of six monkeys remained resistant to infection when challenged with a more pathogenic SHIV strain (SHIV-89.6P). The remaining two animals had reduced viral RNA levels and did not show the precipitous falls in CD4<sup>+</sup> T-cell levels seen in control animals. Interestingly, transfusion of whole blood from one of the protected monkeys (which had remained

negative for plasma viral RNA) facilitated transmission of SHIV infection to a naive animal, suggesting that the protective immune response was controlling, but not entirely eliminating, the virus.

The immunogenicity of prime-boost vaccination against retroviral antigens was further demonstrated in macaques given consecutive inocula of DNA and MVA encoding gag sequences of SIV either as single<sup>12</sup> or multi-epitope<sup>13</sup> constructs. Tetramer binding assays showed that vaccinated macaques had levels of epitope-specific CD8<sup>+</sup> CTLs as high as 1–20% of all circulating CD8<sup>+</sup> T cells. However, most animals were not protected against pathogenic SIV challenge, unlike those in the studies mentioned above, where whole proteins were encoded in both vaccine vectors. A variety of reasons might account for this limited protective efficacy, including the restriction of antiviral responses to CD8<sup>+</sup> CTLs, but not specific CD4<sup>+</sup> Th cells. Nonetheless, these data provide further evidence of the efficacy of prime-boost vaccination for inducing extremely high levels of CMI.

A prime-boost approach has also been shown to generate protective immune

responses in a murine model of malaria infection<sup>14</sup>. The consecutive use of DNA and MVA vectors encoding antigens from *Plasmodium berghei* produced high levels of peptide-specific, IFN- $\gamma$ -secreting CD8<sup>+</sup> T cells, which protected mice against challenge with *P. berghei* sporozoites. This study also clearly demonstrated two important principles of effective prime-boost immunization: (1) the importance of DNA vaccines as priming vehicles and attenuated viruses as boosters; and (2) the nature of the boosting virus. Either reversing the order of immunization or changing the strain of VV from MVA or the closely related NYVAC strain to the WR strain (which replicates extensively in mice), resulted in a failure of protection.

Alternative prime-boost protocols, involving vaccination first with recombinant vectors and boosting with the relevant protein have, despite some reports of protective responses<sup>15</sup>, largely produced disappointing results<sup>16</sup>. Such approaches generally elicit far stronger specific humoral responses than CMI. There are several precedents indicating that reciprocal cross-regulation may occur in the generation of these types of immune responses<sup>17,18</sup>.

**Table 1. Features of DNA vaccines and attenuated poxvirus vectors that might contribute to the efficacy of prime-boost vaccination**

Vector	Property	Immunological consequences
DNA vaccine	Internalized by APCs and encoded proteins delivered to MHC class I and II antigen-processing pathways Contains dinucleotide (CpG) motifs Heterologous vaccine antigen is the only protein expressed Low-level and persistent expression of protein	Efficient induction of CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells Adjuvant for CMI Immune response focused on vaccine antigen (i) Prolonged immune stimulation (ii) Induction of high-affinity T cells
Attenuated poxvirus vector	Expression of vaccine antigens in infected cells facilitates their efficient delivery to MHC class I and II antigen-processing pathways Induces strong production of proinflammatory cytokines (e.g. type I interferons) Lacks genes encoding proteins (normally present in poxviruses) that block host antiviral activity Limited or no replication in mammalian cells  Higher levels of expression of encoded protein	Expansion of T-cell responses induced by DNA vaccination Adjuvant for CMI Greater levels of CMI generated against vector-encoded antigens (i) Immune response largely focused on vaccine antigen (ii) Safe for human use Expansion of high-affinity T cells primed by DNA vaccine

Abbreviations: APC, antigen-presenting cells; CMI, cell-mediated immunity; MHC, major histocompatibility complex.

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It is therefore possible that the development of Th2-driven humoral immunity diminishes the magnitude and/or quality of any CMI response to the vaccine antigen. The efficacy of the DNA/FPV and DNA/MVA prime-boost strategies may consequently be due to their capacity to induce high levels of CMI with little evidence of Th2-driven systemic antibody responses.



### Why is prime-boost vaccination so efficient?

Several features of the vectors used in prime-boost vaccination may underlie their capacity to induce enhanced immune responses, particularly CMI (Table 1). The bacterial plasmid backbone of the DNA vector itself contains short, dinucleotide sequences that strongly stimulate interleukin 12 (IL-12) production and may thus favor the development of CMI responses against encoded vaccine antigens. Another critical element in the ability of DNA vaccines to prime T cells for greatly enhanced secondary responses may be their relatively low-level, but persistent, expression of immunogenic proteins *in vivo*. The phenomenon of affinity maturation is well established for B-cell responses, with steadily increasing antibody affinities arising after multiple exposures to antigen. Recent studies have also demonstrated that T cells may display higher average affinities for major histocompatibility complex (MHC)-peptide molecules after multiple exposures to antigen, and that limiting doses of antigen may select for T cells with receptors of increased affinity<sup>19,20</sup>. Although the relative affinities of T cells induced by different vaccination strategies have not been widely studied, the efficiency of prime-boost immunization may be due, in part, to the ability of DNA vaccines to generate T cells of high affinity, which are expanded following boosting with nonreplicating viral vectors. The nonreplicating nature of these vectors, particularly DNA vaccines and FPV, also means that any immune response is likely to be focused almost entirely on encoded vaccine antigens. This is not the case with many other delivery systems, where reactivity to a plethora of vector antigens may modulate desired responses to the heterologous immunogen.

### Concluding remarks

Prime-boost vaccination strategies, using DNA vaccines and attenuated viral vectors, generate impressive CMI against a variety of encoded antigens. The most effective of these strategies elicit high levels of both CD4<sup>+</sup> and CD8<sup>+</sup> T-cell-mediated immunity, responses currently viewed as crucial for protection against a variety of diseases, including HIV.

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